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## Non-cloning amplification of specific DNA fragments from whole genomic DNA digests using DNA 'indexers'.

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A highly systematic, non-cloning method of distinguishing and isolating every fragment in a class-IIS or interrupted palindrome restriction digest has been developed in our laboratory. These enzymes produce informative, non-identical cohesive ends which can be selectively modified by ligation to individual synthetic oligodeoxyribonucleotides with the corresponding complementary ends. In this way, polymerase chain reaction and sequencing primer sites and labels can be introduced specifically into a single fragment in a total genomic digest. Known and unknown fragments from genomes of the complexity of *Escherichia coli* can be isolated directly in sequencable form without the necessity of synthesizing unique primers. Human DNA has also been assessed in this way. Problems intrinsic to cloning (selective fragment loss, mutation and sequence rearrangement) are avoided. Systematic characterization of DNA fragments by their cohesive ends and length provides tremendous power and flexibility for analysis of any DNA molecule without specific clones, probes or libraries. We report proof of principle of this remarkable system and indicate potential applications in DNA sequence tagged site and restriction mapping, sequencing, restriction-fragment-length polymorphism analysis and DNA diagnostics.

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